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Antibiotic resistance in *Staphylococcus aureus* colonising the intestines of Swedish infants

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ABSTRACT

Staphylococcus aureus has become a frequent coloniser of the intestinal tract of infants, but the health effects of such colonisation are not clear. In this study, the antibiotic resistance patterns of 116 *S. aureus* strains from the commensal intestinal microflora were determined. The strains were obtained from 81 Swedish infants who had been followed with regular stool samples and registration of antibiotic usage during their first year of life. The faecal population levels of the individual strains and the duration of their persistence in the microflora had been determined previously. The prevalence of antibiotic resistance among the 116 strains was modest: methicillin, 0%; penicillin G, 78%; erythromycin A, 3%; tetracycline, 2%; clindamycin, 0.9%; and fusidic acid, 0.9%. Colonisation by antibiotic-resistant strains was unrelated to antibiotic consumption by individual infants. Antibiotic-resistant strains were as capable of persisting in the intestinal microflora and reaching high faecal population levels as fully susceptible strains. No strain lost or acquired resistance during the colonisation period. Thus, antibiotic-resistant strains of *S. aureus* seem to be as fit for competition in the large bowel microflora as susceptible strains, even in the absence of selective pressure from antibiotics. This may aggravate the ecological consequences of antibiotic resistance development.

Keywords Antibiotic resistance, commensal microflora, infants, intestine, selection, *Staphylococcus aureus*

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INTRODUCTION

Staphylococcus aureus is a common cause of septicaemia and soft tissue infections, but is also a member of the normal skin flora. In addition, *S. aureus* frequently colonises the intestinal tract of Swedish infants, often persisting for several months in the intestinal microflora of individual infants, seemingly without untoward effects on their health [1].

Staphylococci are inherently susceptible to most antibiotics, except those with a purely Gram-negative spectrum. However, β-lactamase production evolved rapidly in *S. aureus*, and >50% of hospital-acquired *S. aureus* isolates were penicillin G-resistant by 1948, with this proportion now reaching 80–90% [2]. As new antibiotics were introduced to the market, e.g., tetracycline, streptomycin, erythromycin and gentamicin, *S. aureus* isolates resistant to these antibiotics appeared. Methicillin, a penicillin resistant to *S. aureus* β-lactamase, was introduced into clinical practice in 1960 [2], but the first resistant strain was identified 1 year later. Methicillin-resistant *S. aureus* strains have spread with unanticipated speed in many countries [3]. In 2003, two clinical *S. aureus* isolates were identified that carried the *vanA* gene conferring resistance to vancomycin [4–6].

The normal microflora colonising the skin and mucous membranes may be an important reservoir for antibiotic-resistant bacterial strains, and transfer of resistance elements may also occur in these complex microbial communities. For example, methicillin-resistant *S. aureus* may arise *de novo* in community strains through horizontal acquisition of the *mecA* gene [7]. Despite this, the ecological role of antibiotic resistance and the risk of transfer of resistance elements between strains in the normal microflora have rarely been studied.

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In the present study, intestinal *S. aureus* strains from 81 Swedish infants were tested for resistance to a range of commonly used antibiotics. Stool samples from these infants had been obtained regularly over the first year of life and cultured quantitatively for *S. aureus* in a study examining the role of infant intestinal colonisation pattern on allergy development [1,8]. As all medical treatments had been registered, colonisation by resistant strains could be correlated with antibiotic consumption. Furthermore, all *S. aureus* isolates from each individual infant had been characterised by random amplified polymorphic DNA analysis to determine their strain identity. Persistence in the microflora and faecal population numbers of each strain had been determined and could be compared between resistant and susceptible *S. aureus* strains. Thus, the effect of antibiotic resistance on the fitness of *S. aureus* in the colonic microflora could be assessed.

MATERIALS AND METHODS

Infants and *S. aureus* isolates

In total, 116 *S. aureus* strains were obtained from 81 healthy Swedish infants born between 1998 and 2002 at the Sahlgrenska University Hospital ($n = 78$) or Varberg Hospital ($n = 3$) in south-west Sweden. These infants were in a cohort of 120 infants who harboured *S. aureus* in their intestinal microflora at least once during their first year of life. The cohort was recruited for a previous study [1,8], and the *S. aureus* colonisation pattern of the cohort has been described previously [1]. The sampling schedule consisted of rectal swab cultures taken at an age of 3 days, and quantitative cultures of infants' stools at the ages of 1, 2, 4 and 8 weeks, and 6 and 12 months. *S. aureus* was isolated and identified to the strain level as described previously [1].

One or more toxins was produced by 36% of the strains, as determined by reversed passive latex agglutination (Oxoid, Basingstoke, UK). Staphylococcal enterotoxins A, B, C and D were produced by 12, 2, 23 and 2 strains, respectively. Toxic shock syndrome toxin 1 was produced by 17 strains.

The infants' feeding patterns, health status and antibiotic consumption were registered by the parents in a diary, and these details were recorded after 6 and 12 months by a study nurse via a telephone interview. Informed consent was obtained from the parents, and the study was approved by the Medical Ethics Committee of Göteborg University.

Antibiotic susceptibility testing

Antibiotic susceptibility was tested by the agar disk diffusion method [9]. *S. aureus* isolates were cultivated overnight on blood agar plates. Five to ten colonies were picked with a sterile loop and suspended in 2 mL of phosphate-buffered saline to a density of 0.5× McFarland standard. This was further diluted 1:100 and spread on PDM antibiotic sensitivity testing medium agar plates using a cotton-tipped swab (AB Biodisk, Solna, Sweden). The following antibiotic-containing disks were applied: penicillin G (10 μg), vancomycin (5 μg), teicoplanin (30 μg), erythromycin A (15 μg), tetracycline (30 μg), clindamycin (15 μg), gentamicin (30 μg), tobramycin (30 μg), ciprofloxacin (10 μg), trimethoprim (5 μg), fusidic acid (50 μg), and chloramphenicol (30 μg) (AB Biodisk). After the plates had been incubated at 37°C for 16–20 h, the zone diameters were measured and the isolates were defined as sensitive, intermediate or resistant [9]. For methicillin, a bacterial suspension corresponding to 0.5× MacFarland standard was inoculated on to PDM blood agar plates, and an oxacillin disk (1 μg) was applied. Zone diameters were measured after incubation at 30°C for 24 h. Isolates with penicillin G zones of >30 mm were tested for β-lactamase production using the nitrocefin test (Oxoid); isolates producing β-lactamase were defined as penicillin-resistant. MICs were determined by Etest [10] for isolates that were not fully susceptible to erythromycin A, tetracycline or clindamycin by the disk diffusion method. Etest strips (AB Biodisk) were placed on PDM agar plates inoculated with a bacterial suspension (0.5× McFarland standard) and incubated for 20 h at 37°C.

PCR experiments

All isolates were tested for presence of the *mecA* gene with the primer set MecA1 (5′-GTAGAAATGATCGAACGTCCGATAA-3′) and MecA3 (5′-CCAATTCCACATTGTTTCGGTCTAA-3′) (Scandinavian Gene Synthesis, Köping, Sweden) [11]. Bacterial DNA was released using the modified InstaGene protocol (Bio-Rad, Richmond, CA, USA). PCR reactions contained 4 μL DNA, 10 pmol of each primer, 0.1 mmol dNTPs, 3.0 mmol $MgCl_2$, and 2 U *Taq* polymerase. Thermal cycling parameters comprised 4 min at 94°C, followed by 30 cycles of 45 s at 94°C, 45 s at 62°C and 2 min at 72°C. The 310-bp *mecA* product was detected by electrophoresis on agarose 1.8% w/v gels, followed by ethidium bromide staining and examination under UV light.

Erythromycin-resistant isolates were examined by PCR for the presence of the methylase genes *ermA*, *ermB*, *ermC* and *ermTR* and the efflux pump genes *msrA* and *mefA/*E as described previously [12].

RESULTS

Antibiotic resistance in *S. aureus* strains

The antibiotic resistance patterns of the 116 *S. aureus* strains are shown in Table 1; 78% of the strains were resistant to penicillin. Five strains were resistant to penicillin G and at least one other antibiotic: erythromycin A and clindamycin (one strain), erythromycin A and tetracycline (one), erythromycin A (one), tetracycline (one) and fusidic acid (one).

Strains defined as resistant to erythromycin A, tetracycline or clindamycin by the disk diffusion method exhibited MICs of these antibiotics that exceeded those obtained when testing strains lacking resistance mechanisms, as defined by the

Table 1. Frequency of resistance to antibiotics in 116 *Staphylococcus aureus* strains colonising the intestine of 81 healthy Swedish infants

Antibiotic	Infants carrying resistant strains		Number of resistant strains		MIC (mg/L)[a]
	n	%	*n*	%	
Penicillin G	69	85	91	78	ND
Erythromycin A	3	4	3	3	256
Tetracycline	2	3	3	2	8–24
Clindamycin	1	1	1	0.9	256
Fusidic acid	1	1	1	0.9	ND
Oxacillin	0	0	0	0	–
Vancomycin	0	0	0	0	–
Teicoplanin	0	0	0	0	–
Gentamicin	0	0	0	0	–
Tobramycin	0	0	0	0	–
Ciprofloxacin	0	0	0	0	–
Trimethoprim	0	0	0	0	–
Chloramphenicol	0	0	0	0	–

ND, not determined.
[a]MICs for resistant isolates were determined by Etest.

Swedish Reference Group for Antibiotics (Table 1) [9].

There were no significant differences in antibiotic resistance between toxin-producing and non-toxin-producing strains (Fisher's exact test, data not shown).

Antibiotic resistance and fitness in the microflora

The population counts of each *S. aureus* strain had been determined previously [1]. The average population counts of strains fully susceptible to all antibiotics ($n = 25$), resistant to penicillin G only ($n = 86$), or resistant to penicillin G and at least one other antibiotic ($n = 5$), were compared. The stool counts of *S. aureus* decrease with age as a more complex flora develops [1], but antibiotic-resistant and -sensitive strains had similar average population numbers at each time-point (Fig. 1).

Seventy-eight *S. aureus* strains persisted in the intestinal microflora of an infant for an average of 17 weeks [1]. Several isolates of these strains were available, and the antibiotic resistance patterns of the first and last isolate were determined. If the strain was resistant to any antibiotic in addition to penicillin G ($n = 5$), the antibiotic resistance patterns of all isolates were determined. In each case, all tested isolates of a single strain showed identical patterns of antibiotic resistance.

Strains that colonised an infant for ≥3 weeks were defined as resident, while those colonising for shorter periods were defined as transient [13]. In the present study, 78 strains were defined as

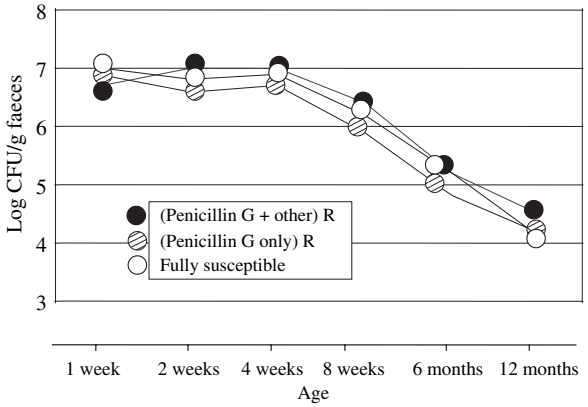

Fig. 1. Faecal population counts of *Staphylococcus aureus* isolated from infant stools collected at different time-points. The mean population counts at different infant ages of strains resistant to penicillin G only, of strains resistant to penicillin G and other antibiotics, and of fully sensitive strains, are shown. Quantitative cultures were performed from fresh stools on staphylococcus agar as described previously [1].

Table 2. Frequency of resistance to penicillin G (PcG) or other antibiotics in resident and transient intestinal *Staphylococcus aureus* strains from Swedish infants

	n	Resistant (%)		Fully susceptible (%)
		Only PcG	PcG + other	
Resident strains[a]	78	78	7	15
Transient strains	19	67	0	33

[a]Strains persisting in the microflora of an infant for >3 weeks were termed resident; strains persisting for shorter periods were termed transient.

resident and 18 as transient, while strains that occurred only at 2, 6 or 12 months could not be classified because of the long sampling intervals.

Resistance to antibiotics (penicillin G only, or penicillin G plus at least one other antibiotic) was more common among resident than transient strains, but the difference did not reach statistical significance (p 0.09) (Table 2). All five strains that were resistant to penicillin and at least one other antibiotic were resident in the microflora.

Antibiotic consumption and resistance

Seventeen (21%) of the 81 infants had been treated with antibiotics during their first year. Six of these received more than one type of antibiotic. Ten infants received penicillin V, nine amoxycillin, two trimethoprim, and one infant each erythromycin, penicillin G or fusidic acid.

Sixteen infants received β-lactam antibiotics, and the resistance patterns of strains from these

Table 3. Frequency of penicillin-resistant strains in the intestinal microflora in relation to treatment with β-lactam antibiotics

		No. of strains (%)	
	No. of strains	Penicillin-resistant	Penicillin-sensitive
Non-β-lactam-treated children (n = 65[a])	92	73 (79)	19 (21)
β-Lactam-treated children (n = 16[b])	24		
Strains found before β-lactam-treatment	16	12 (75)	4 (25)
Strains found after β-lactam-treatment	3	2 (67)	1 (33)
Strains found before and after treatment	5	4 (80)	1 (20)

[a]One child was treated with erythromycin, and the others received no antibiotics.
[b]Penicillin V (ten children), amoxycillin (nine children), or penicillin G (one child).

infants and strains from infants who did not receive β-lactam antibiotics are shown in Table 3. There was no difference in penicillin resistance between these groups of strains (Table 3). One infant received penicillin V between 6 and 12 months, and the same penicillin-sensitive strain was isolated at both time-points. However, it is unclear whether the strain persisted during treatment, or whether the child was recolonised with the same strain, e.g., from household contacts.

Among the five strains that were resistant to both penicillin G and at least one other antibiotic, three were found in infants who did not receive antibiotics. Two strains were found in infants who received ampicillin, or tobramycin and trimethoprim, respectively, but the strains had been established in the microflora before treatment. Moreover, the resistance patterns of the strains (erythromycin A and clindamycin, and erythromycin A and tetracycline, respectively) were not related to the antibiotics received.

Resistance genes

None of the 116 strains analysed was *mecA*-positive. Of the three isolates that were resistant to erythromycin, one was positive for the *ermA* gene, and one for the *ermC* gene. The third isolate was negative for all genes tested, but the resistance phenotype was suggestive of *erm* gene carriage.

DISCUSSION

S. aureus is both an important pathogen and a common member of the commensal flora. In the present study, commensal *S. aureus* strains isolated from the intestinal flora of healthy Swedish infants were assayed for susceptibility to a range of commonly used antibiotics. *S. aureus* colonises the intestines of 75% of Swedish infants during the first year of life [1], and assessment of these resistance patterns may give an insight into the ecological consequences for *S. aureus* of antibiotic treatment of individuals and antibiotic usage in society at large.

The strains isolated were resistant to penicillin G (78%), erythromycin (3%), tetracycline (2%), clindamycin (0.9%) and fusidic acid (0.9%), but not to methicillin. These figures were similar to or slightly lower than those reported with clinical *S. aureus* isolates in Sweden for erythromycin (3.8%), clindamycin (1.9%), and methicillin (0.7%) [9]. Resistance to fusidic acid was more common among clinical isolates (9.5% in 2002), which may relate to clonal spread in Sweden of fusidic acid-resistant *S. aureus* causing superficial infections [14]. These clinical isolates were from a cross-selective sample from adults and children throughout Sweden. To our knowledge, no comparisons have been made between *S. aureus* isolates deriving from adults and children. However, as strains colonising infant intestines usually derive from the parents' skin flora [8], the *S. aureus* tested in the present study may represent commensal isolates from healthy people in a Swedish urban population.

As antibiotic-resistant strains appeared in the microflora without any obvious link to antibiotic treatment of the individual infant, the antibiotic policy of society at large may be more important than the antibiotic consumption of the individual in this context. Antibiotic consumption in Sweden is lower than in the UK (13.5 vs. 18.0 daily doses/1000 inhabitants/day) [15], and commensal *S. aureus* strains isolated from healthy British children by mouthwash were more resistant compared to our findings: erythromycin (6%), tetracycline and chloramphenicol (3% each), and methicillin (2%) [16]. Similarly, 2.5% of *S. aureus* isolates from the nares of healthy children in Chicago, USA were methicillin-resistant [17].

The infants in the present study were followed longitudinally with regular sampling of the microflora. Consecutive isolates of persisting strains were examined and all were found to maintain their antibiotic susceptibility or resistance patterns over the entire colonisation period. Furthermore, resistant strains persisted in the intestinal microflora for as long, and reached similar population levels, as fully sensitive

strains. Thus, resistance to antibiotics did not seem to impede the survival fitness of *S. aureus* in the intestinal commensal microflora. Strains that have acquired resistance in one host may therefore spread to other hosts unhindered by their resistance phenotype. This points to the need to minimise the total antibiotic load in society.

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